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U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.51)

Unassigned

09/529442

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

INTERNATIONAL APPLICATION NO.
PCT/SE98/01717INTERNATIONAL FILING DATE
24 September 1998PRIORITY DATE CLAIMED
23 October 1997

412 Recd PCT/PTO 13 APR 2000

TITLE OF INVENTION
ENCAPSULATION METHOD

APPLICANT(S) FOR DO/EO/US

Timo Laakso and Mats Reslow

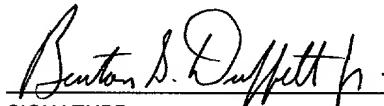
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. has been transmitted by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US)
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. A FIRST preliminary amendment.
- A SECOND or SUBSEQUENT preliminary amendment.
14. A substitute specification.
15. A change of power of attorney and/or address letter.
16. Other items or information:

A certified copy of the priority application, Sweden Patent Application No. 9703874-9, filed 23 October 1997, was duly filed in connection with PCT/SE98/01717 and was received by DO/EO/US. Thus, it is believed that the priority claim has been properly substantiated.

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50) Unassigned 09/529442		INTERNATIONAL APPLICATION NO. PCT/SE98/01717	ATTORNEY'S DOCKET NUMBER 003300-634
17. <input type="checkbox"/> The following fees are submitted:		CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO \$840.00 (970) International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00 (956) No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$760.00 (958) Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$970.00 (960) International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$96.00 (962)			
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$ 970.00	
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)). 20 <input type="checkbox"/> 30 <input type="checkbox"/>		\$ 0.00	
Claims	Number Filed	Number Extra	Rate
Total Claims	22 -20 =	0	X\$18.00 (966) \$ 36.00
Independent Claims	1 -3 =	0	X\$78.00 (964) \$ 0.00
Multiple dependent claim(s) (if applicable)		+\$260.00 (968) \$ 0.00	
TOTAL OF ABOVE CALCULATIONS =		\$ 1,006.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).		\$ 0.00	
SUBTOTAL =		\$ 1,006.00	
Processing fee of \$130.00 (156) for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)). 20 <input type="checkbox"/> 30 <input type="checkbox"/> +		\$ 0.00	
TOTAL NATIONAL FEE =		\$ 1,006.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). per property +		\$ 40.00	
TOTAL FEES ENCLOSED =		\$ 1,046.00	
		Amount to be: refunded \$	
		charged \$	
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1,046.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u> . A duplicate copy of this sheet is enclosed.			
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.			
SEND ALL CORRESPONDENCE TO:			
Benton S. Duffett, Jr. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404			
 SIGNATURE			
<u>Benton S. Duffett, Jr.</u> NAME			
<u>22,030</u> REGISTRATION NUMBER			

Patent
Attorney's Docket No. 003300-634

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
Timo Laakso and Mats Reslow) **BOX PCT**
Application No. (corresponds to PCT/SE98/01717))
Filed: April 13, 2000) Attn: DO/EO/US
For: ENCAPSULATION METHOD) Group Art Unit: Unassigned
) Examiner: Unassigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This application corresponds to International Application No. PCT/SE98/01717.

Please amend the above-identified application, filed concurrently herewith, as indicated.

In the Abstract of the Disclosure

Please add the Abstract of the Disclosure that is provided on a separate sheet.

In the Claims

Claim 3, line 1, delete "any one of claims 1 and 2" and insert --claim 1--.

Claim 4, lines 1 and 2, delete "any one of claims 1, 2 and 3" and insert --claim 1--.

Claim 6, lines 1 and 2, delete "any one of the preceding claims" and insert --claim 1--.

Claim 7, lines 1 and 2, delete "any one of the preceding claims" and insert --claim 1--.

Claim 8, lines 1 and 2, delete "any one of the preceding claims" and insert --claim 1--.

Claim 9, lines 1 and 2, delete "any one of the preceding claims" and insert --claim 1--.

Claim 11, lines 1 and 2, delete "any one of the preceding claims" and insert
--claim 1--.

Claim 12, lines 1 and 2, delete "any one of the preceding claims" and insert
--claim 1--.

Claim 13, lines 1 and 2, delete "any one of the preceding claims" and insert
--claim 1--.

Claim 15, line 1, delete "any one of claims 1-12" and insert --claim 1--.

Claim 16, line 1, delete "any one of claims 1-12" and insert --claim 1--.

Claim 17, lines 1 and 2, delete "any one of the preceding claims" and insert
--claim 1--.

Claim 18, lines 1 and 2, delete "any one of the preceding claims" and insert
--claim 1--.

Claim 19, lines 1 and 2, delete "any one of the preceding claims" and insert
--claim 1--.

Claim 21, lines 3 and 4, delete ", obtainable by a method according to any one of
claims 1 to 20" and insert --obtained by the method of claim 1--.

REMARKS

The present Amendment adds an Abstract of the Disclosure and eliminates the use of
multiple dependency in the claims.

Preliminary Amendment
Attorney's Docket No. 003300-634
Page 3

The examination and allowance of the Application are respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

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Date: April 13, 2000

ENCAPSULATION METHODFIELD OF INVENTION

The present invention is within the field of encapsulating active substances, e.g. drugs, in biodegradable polymers. More specifically the invention relates to a novel advantageous encapsulation method which is suitable for water soluble as well as water insoluble active substances and which gives highly active micro as well as nanoparticles with high encapsulation efficiency.

10

BACKGROUND OF THE INVENTION

The encapsulation of materials may provide beneficial properties. For example, drugs that are encapsulated may provide increased stability, longer duration of action and increased efficiency. For convenience drugs are often encapsulated in solid materials which have a size suitable for injection, that is generally below 200 μm in diameter, and then the process is referred to as a micro-encapsulation.

20

Microencapsulation processes may yield microcapsules, microspheres or microparticles. Microcapsules consist of a core and a shell that covers the core. The core may be composed of another polymer than the shell or of another material altogether, e.g. of the active substance itself. The active substance is generally located in the core but may also be located in the outer shell. Microspheres are spherical in shape and have a more homogenous matrix. Microparticle is a more general term than microspheres in that it is not restricted to spherical shapes. Sometimes it can be difficult to distinguish between microcapsules, microspheres and microparticles, and the term microparticles will be used herein with reference to all three classes.

30

Methods of preparing microparticles in the prior art have been described extensively in both the patent and

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TO THIS DOCUMENT

the scientific literature (see e.g. Jalil R, Nixon JR. Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. *J Microencapsul* 5 1990;7:297-325). They may generally be classified in three types, which are exemplified below in connection with the preparation of microspheres of poly(lactide-co-glycolide) (PLGA). PLGA is a well accepted polymer for preparing sustained release microspheres and often the 10 first choice for preparing biocompatible microspheres intended for parenteral administration in humans. Said polymer is not soluble in water.

1. Phase separation techniques using coacervating agents, or non solvents, such as mineral oils and vegetable oils. The active substance, e.g. a polypeptide is first dissolved in the aqueous phase of a water-in-oil emulsion. The polypeptide can also be dispersed directly in the polymer phase as a fine powder. Polymer is precipitated either around the aqueous droplets, or on the 15 polypeptide powder, by the addition of a non-solvent for the polymer, such as silicon oil. Then a hardening agent is added to extract the organic solvent from the microspheres. The main disadvantage with said process is the large amount of organic solvent needed for extraction and 20 for washing. The previously used hardening agents including freons, hexane, heptane, cyclohexane and other alkane solvents leave substantial amounts of hardening agents residues in the microspheres and/or necessitate extensive procedures for removing the solvent. Often very large 25 amounts of the second organic solvent are needed and they are often undesirable for health, economical and environmental reasons. Examples in the prior art include heptan (EP 0 052 510), aliphatic fluorinated and fluorohalogenated hydrocarbons sold as FREONS (SE 462 780), and other 30 (US 5,000,886). A further drawback when using e.g. an alkane hardening solvent is that it is flammable. Another 35 drawback is the impact thereof on the environment.

2. Spray drying and spray coating

In spray drying the polymer and the drug are mixed together in a solvent for the polymer. The solvent is then evaporated by spraying the solution. This results in 5 polymeric droplets containing the drug. However, sensitive substances such as proteins can be inactivated during the process due to the elevated temperatures used and the exposure to organic solvent/air interfaces. Further 10 disadvantages include generation of high porosity due to rapid removal of the organic solvent. A variation that has been introduced to avoid these shortcomings is the use of low temperature during microsphere formation (US 5,019,400, WO 90/13780 and US 4,166,800). Microcapsules 15 have been prepared using spray coating of drug-containing microparticles with PLGA polymers (US 4,568,559).

3. Solvent evaporation

In solvent evaporation techniques the polymer is dissolved in an organic solvent which contain the dispersed active drug, the solution then being added to an 20 agitated aqueous outer phase which is immiscible with the polymer. The aqueous outer phase usually contains surfactants to stabilise the oil-in-water emulsion and to prevent agglomeration. The emulsifier used is typically 25 polyvinylalcohol. Emulsifiers are included in the aqueous phase to stabilise the oil-in-water emulsion. The organic solvent is then evaporated over a period of several hours or more, thereby solidifying the polymer to form a polymeric matrix. The solvent can also be extracted by adding 30 the above mentioned suspension to a large volume of water (US 5,407,609).

The final formulation to be used for pharmaceutical applications, especially for parenteral administration, should consist of discrete, non-agglomerated microspheres with the desired size distribution and containing no 35 toxic or in any other way undesirable substances. In order to obtain preparations having the characteristics described above it is necessary to use emulsifiers. The

emulsifier can serve several purposes: (1) assist in obtaining the correct droplet size distribution of the emulsion; (2) stabilise the oil-in-water emulsion to avoid coalescence of the droplets; and (3) prevent the 5 solidified microspheres from sticking to each other. The most commonly used emulsifier for preparing PLGA microspheres is polyvinyl alcohol. However, since polyvinyl alcohol is listed in the 1976 Register of Toxic Effects 10 of Chemical Substances and is also implicated as carcinogenic when introduced parenterally into animals ("Carcinogenic studies on Water-Soluble and Insoluble Macromolecules", Archives of Pathology, 67, 589-617, 1959) it is considered undesirable for pharmaceutical preparations administered by injection. This problem has 15 been recognized and attempts of replacing polyvinyl alcohol with other emulsifiers can be found in the prior art, for example in US 4,384,975, wherein a carboxylic acid salt surfactant, e.g. sodium oleate was used to stabilise an oil-in-water emulsion. However, despite its drawbacks 20 polyvinyl alcohol is still the most widely used surfactant. However, for the above-mentioned reasons it would be highly desirable to avoid the use of polyvinyl alcohol and other surfactants in microsphere preparations.

Solvent evaporation works well for hydrophobic drugs 25 but for hydrophilic drugs, such as many peptides and proteins, the amount of incorporated drug can be low due to loss of drug to the aqueous phase which is used to extract the organic solvent. Attempts to circumvent this problem include modifying the hydrophilic drug into a 30 less soluble form (WO 96/07399) increasing the viscosity of the inner aqueous solution containing the active drug in a process where a water-in-oil emulsion is first created and the organic solvent then extracted with water (US 4,652,441) and reducing the time available for diffusion 35 (US 5,407,609).

Further, the use of the commonly employed organic solvents, like methylene chloride or ethyl acetate, often

results in loss of biological activity for sensitive drugs. Thus, for instance for proteins the three dimensional conformation which is required for biological activity is often lost. Attempts to circumvent this problem 5 includes modification of the active substance into a more stable form (US 5,654,010 and WO 96/40074) keeping the temperature as low as possible during the process (WO 90/13780), and using different protein stabilisers (US 5,589,167, Cleland JL, Jones AJS, "Development of stable 10 protein formulations for microencapsulation in biodegradable polymers". Proceedings of the International Symposium on Controlled Release of Bioactive Materials 1995;22:514-5). However, proteins are generally sensitive to organic solvents and reducing or eliminating the exposure is highly desirable.

Another disadvantage with the solvent evaporation method is the need for using high shear mixing in order to obtain small microspheres or nanospheres. This may result in degradation or conformational changes of the active substance, especially if it is a protein which is 20 dependent on a three dimensional conformation for its biological activity. The use of high shear mixing is also energy consuming.

In connection with the prior art it can also be 25 added that processes for preparing microspheres from polymers soluble in water are known from e.g. US 4,822,535 and US 5,578,709. In said processes two mutually immiscible aqueous liquid phases are used, of which one is solidified into microspheres. However, as said, 30 these methods cannot be used for the preparation of microspheres from polymers that cannot be dissolved in water.

The present invention relates to a novel method of 35 encapsulating active substances in biodegradable polymers by which the prior art disadvantages are eliminated or at least essentially reduced. For instance the invention makes it possible to obtain high incorporation efficiency

of the active substance in the biodegradable polymer and/or to accomplish smaller microparticles or even nanoparticles containing highly active doses of the active substances. Furthermore, the amounts of organic solvents 5 are highly reduced. As compared to previously used methods the invention also enables a reduction of the energy input required to obtain micro- or nanoparticles.

OBJECTS OF THE INVENTION

10 One object of the invention is to provide a method of preparing controlled or sustained release particles having a high entrapment of water soluble substances, e.g. sensitive drugs, without the use of large volumes of organic solvents.

15 Another object is to provide a method wherein low energy mixing is utilized only, which is also advantageous in connection with sensitive substances.

20 Still another object is to provide a method by which small particle sizes, such as micro or even nano size particles, can be obtained in a simple way.

One other object is to provide a method by which the requirement for using PVA and other surfactants is eliminated.

25 Other objects of the invention should be clear to a person skilled in the art when reading the description above and below.

SUMMARY OF THE INVENTION

30 More specifically the present invention relates to a method of encapsulating an active substance in a biodegradable polymer, which method comprises:

- a) dissolving said biodegradable polymer in an organic solvent therefor;
- b₁) dispersing said active substance in the organic solution obtained in step a), to provide a dispersion with the active substance as the inner phase thereof; or alternatively

b₂) emulsifying said active substance, dissolved in water or other aqueous solvent therefor, in the organic solution obtained in step a), to provide an emulsion with the active substance as the inner aqueous phase thereof;

5 and

c) subjecting the dispersion obtained in step b₁), or alternatively the emulsion obtained in step b₂), to an encapsulation operation with an aqueous polyethylene glycol solution as a continuous phase, so as to obtain

10 micro- or nanoparticles having the active substance encapsulated therein.

Thus, according to one aspect of the invention there is provided a simple method of preparing micro- or nanoparticles containing a sensitive biologically active material, e.g. a protein, while using minimal amounts of organic solvent. It has surprisingly been found possible to replace the normally used organic solvent as the continuous or extraction phase by an aqueous solution of the non-toxic and pharmaceutically acceptable polymer poly-
15 ethylene glycol (polyethylene oxide) as a continuous phase and as an extraction medium.

It has also been found that the uptake of active ingredient into the particles can be markedly improved by said use of polyethylene glycol in water or other aqueous solvent as outer(external) phase. The use of solvent evaporation techniques with an aqueous outer phase often results in poor encapsulation as water soluble polypeptides are distributed also to the external phase, especially when small microspheres are obtained. With the
25 present invention high loading combined with small particle size can be obtained provided that the concentration of polyethylene glycol, and other conditions, are controlled such that the active substance is not distributed to the outer phase.

30 The microparticles can easily be washed and rinsed with water, which is an advantage as compared to the phase separation technique where large amounts of organic

solvents are used. Other surprising findings in connection with the use of polyethylene glycol/aqueous solvent as outer phase is that small sized particles are obtained even with low mixing forces and that no surfactants are
5 needed.

The obtained microparticles are well suited for sustained release purposes and are especially adapted for oral or parenteral administration. When prepared with sizes or diameters of less than 10 μm , and preferably
10 0.5-3 μm , they are also suitable for nasal or pulmonal administration to provide either local or systemic effect.

In addition to the unexpected findings referred to above it should also be noted that polyethylene glycol
15 (PEG) is previously known per se to have unique properties for a variety of biotechnical and biomedical applications, which makes the present invention even more advantageous for biotechnical and biomedical applications.

These unique properties are e.g. summarized in Harris, J.M. (ed) Poly(ethylene glycol) chemistry: biotechnical and biomedical applications. 1992, Plenum Press, New York.

PEG has unique properties of major importance for its use in a variety of biotechnical and biomedical applications. One of these is its outstanding effectiveness in excluding other polymers from the volume it occupies in a water solution, which has been utilised to obtain rejection of proteins e.g. in liposomes and small particles with long circulation times after intravenous injection, hospitability to biological materials, non-immunogenicity and non-antigenicity. Another is the formation of aqueous two-phase systems with other polymers (Per Åke Albertsson, Partition of cell particles and macromolecules. Separation and purification of biomolecules, 20
35 cell organelles, membranes, and cells in aqueous polymer two-phase systems and their use in biochemical analysis and biotechnology. Third Edition, 1986, John Wiley &

Sons). PEG is non-toxic and generally harmless to proteins and cells. Of the numerous applications of PEG can be mentioned: (1) as a co-solvent for some drugs for injection, (2) as a volume-excluder to increase the concentration of e.g. proteins to induce crystallization, (3) as a part of aqueous two-phase systems used for e.g. purification of biological materials under mild conditions, (4) induction of cell fusion to obtain e.g. hybridomas used for production of monoclonal antibodies, (5) covering the surface of e.g. liposomes and nanoparticles to increase their residence time in the circulation, and (6) covalent attachment of PEG to proteins to obtain conjugates which are still biologically active but no longer immunogenic and antigenic; such PEG-protein adducts having been approved for parenteral use in humans.

PEGs are also sometimes referred to as poly(ethylene oxide) PEO, poly(oxyethylene) and polyoxirane. In general usage, poly(ethylene glycol) refers to molecular weights below 20000, and poly(ethylene)oxide refers to higher molecular weights polymers. In other words the term polyethylene glycol as used in connection with the invention covers also poly(oxyethylene) and polyoxirane.

These objects as well as other objects and advantages of the present invention will become apparent to those skilled in the art from the description following below.

DETAILED DESCRIPTION OF THE INVENTION

The active substances to be used in the method of the invention are preferable biologically active substances, e.g. drugs, such as proteins, peptides, polypeptides, polynucleotides, oligonucleotides, plasmides or DNA. Examples of protein drugs are growth hormone, erythropoietin, interferon(α, β, γ -type), vaccines, epidermal growth hormone and Factor VIII. Examples of peptide drugs are LHRH analogues, insulin, somatostatin, calcitonin, vasopressin and its derivatives.

In the case of proteins they can also be complexed with various substances, e.g. metals, amino acids, salts, acids, bases and amines, to decrease solubility or increase stability. They can further be prepared in the 5 form of a pro-drug or PEG can be attached e.g. to the proteins to increase solubility or stability, modify pharmacokinetics or reduce immunogenicity.

Examples of non-protein drugs suitable for use in the method of the invention can be found for example in 10 the following groups:

anti-tumor agents, antibiotics, anti-inflammatory agents, 15 antihistamines, sedatives, muscle relaxants, antiepileptic agents, antidepressants, antiallergic agents, bronchodilators, cardiotonics, antiarrythmic agents, vasodilators, antidiabetic agents, anticoagulants, hemostatics, 20 narcotic agents and steroids.

The active substances which can be encapsulated in accordance with the method claimed are, however, not restricted to biologically active substances, non-biological substances can be encapsulated, e.g. pesticides, 25 fragrances, flavouring agents, catalysts and herbicides.

The proper amount of active substance to be encapsulated is dependent on type of substance, duration time 25 and desired effect, and is of course controlled to an amount that is in each specific case encapsulable by the method according to the invention. Generally said amount is chosen within the range of about 0,001% to 90%, preferably about 0,01 to 70%, more preferably about 0.1 to 30 45%, and most preferably about 0.1 to 40%, said percentages being by weight based on the weight of the final particles.

In the case of a drug the substance can be used per se or in the form of a pharmaceutical salt. When the drug 35 has a basic group, such as amino groups, it can form salts with carbonic acid, hydrochloric acid, sulphuric acid, acetic acid, citric acid, methanesulfonic acid or

the like. When the drug has an acidic group, such as a carboxyl group, it can form salts with metals (e.g. Ca^{2+} , Zn^{2+}), organic amines (e.g. ethanolamine) or basic amino acids (e.g. arginine). The drug can further be precipitated using various means, optionally followed by size reduction, such as precipitation with divalent metals (e.g. Ca^{2+} , Zn^{2+}). The drug may also be crystallized.

10 The biodegradable polymer that can be used in the present invention is not limited to any specific material as long as it can be dissolved in an organic solvent and is slightly soluble or insoluble in the outer phase, e.g. poly(ethylene glycol)/aqueous phase and is otherwise suitable for the preparation of sustained release micro- or nanoparticles.

15 Preferably the biodegradable polymer used in the method claimed has a weight average molecular weight in the range of about 2000 to 200000, more preferably about 2000 to 110000.

20 Examples of biodegradable polymers are polyesters, poly- β -hydroxybutyric acid, polyhydroxyvaleric acid, polycaprolactone, polyesteramides, polycyanoacrylates, poly(amino acids), polycarbonates and polyanhydrides.

25 A preferred biodegradable polymer is an aliphatic polyester, e.g. homo or copolymers prepared from α -hydroxy acids, preferably lactic acid and glycolic acid, and/or cyclic dimers of α -hydroxy acids, preferably lactides and glycolides.

30 When lactic acid/glycolic acid are used as the above-mentioned polymers, the composition or weight ratio (poly)lactic acid/(poly)glycolic acid is preferably about 99/1 to 35/65, more preferably 95/5 to 50/50. They may be used in the form of a copolymer or a mixture of these two or more polymers. The exact composition of the polymer depends on the desired release kinetics, especially the 35 duration of release.

The organic solvent used in step A can be any solvent that is capable of forming an emulsion with a wa-

PCT/SE98/01717

ter/PEG mixture, can be removed from the oil droplets through said water/PEG mixture and is capable of dissolving the biodegradable polymer. In other words the solvent should be immiscible, or essentially immiscible, but

5 slightly, or very slightly, soluble in said water/PEG mixture. Examples of suitable solvents are ethyl acetate, dichloromethane, methyl ethyl ketone and methyl isobutyl ketone. These solvents can be used alone or in combinations.

10 The inner aqueous phase may contain agents for controlling the stability and, if desired, the solubility of the biologically active substance. Such agents may be pH controlling agents and stabilizers for drugs or other active substances.

15 As can be gathered from the above-mentioned the method according to the invention can be utilized to encapsulate water soluble as well as water insoluble active substances.

20 Examples of embodiments of these two cases will now be presented below.

The encapsulation method, as exemplified by a water soluble drug, such as a peptide or protein drug can comprise the following steps. The drug solution is prepared in any conventional way and optionally while using pH

25 controlling or drug stabilizing agents. This aqueous solution of the drug, which is to form the inner aqueous phase, is poured into an external (oil) phase containing a biodegradable polymer dissolved in a suitable organic solvent and the mixture is emulsified to provide a W/O

30 emulsion. The emulsification can be prepared using conventional emulsification techniques, such as, propeller mixing, turbine mixing, ultrasonication or use of static mixers.

If the active substance is to be dispersed directly

35 in the polymer solution, without being dissolved in water, the drug should have a suitable particle size. A suitable particle size is about 0.5-20 μm , preferably

0.5-10 μm , such as 0.5-3 μm . Otherwise, the dispersion step can be carried out as described above for the emulsification step.

The resulting W/O emulsion/dispersion is then 5 ejected to an encapsulation operation. The W/O emulsion/dispersion is added to an aqueous solution containing polyethylene glycol. The polyethylene glycol/aqueous solution is stirred during the addition of the active substance/polymer solution. The W/O emulsion/dispersion 10 can also be mixed with the polyethylene glycol solution by using motionless mixers.

Typically the molecular weight of the polyethylene glycol is within the range of about 1000 to 40000 Da, 15 preferably 5000 to 35000 Da. Depending on said molecular weight, and the properties of the substance to be encapsulated, the concentration of polyethylene glycol is controlled within the range of 20-80% (w/w), preferably 20-60% (w/w), such as 30-55% (w/w) or 30-50% (w/w). In other words a relatively high PEG concentration is used in the 20 outer phase, to obtain a stable emulsion and to prevent diffusion of active ingredient from the droplets/particles. The determination of the optimal concentration can be made by experimentation that is relatively straightforward to someone skilled in the art.

25 The particles thus formed are generally collected by centrifugation or filtration and rinsed with distilled water or suitable aqueous buffers, several times to remove the excess of polyethylene glycol from the surfaces. To prevent aggregation during the washing and drying procedure, mannitol, Tween 80, or other suitable substances, may be added to the rinsing water. The particles 30 thus obtained can then be dried by conventional means, for instance in vacuum or by a streaming nitrogen gas flow or by lyophilization or air suspension drying.

35 The particle sizes of the particles obtained by the invention are dependent on the desired uses of said particles as is well known within this technical field.

Thus, for instance, when the particles are intended for injection, the particle size should satisfy the dispersibility and needle passage requirements. Furthermore, the particles can be handled or treated in any manner previously known to a person skilled in the art. Thus, a controlled release injectable preparation of said particles can e.g. be dispersed with a suspending agent, containing e.g. mannitol, polysorbate 80, or sodium carboxymethylcellulose.

10 Other embodiments of the method according to the invention are defined in sub-claims or in the Examples presented below.

15 According to a second aspect of the invention there are also provided sustained release micro or nanoparticles per se containing an active substance encapsulated in a biodegradable material, which particles are obtainable by a method as claimed in any one of the method claims.

20 Thus, preferable embodiments thereof are the same as those embodiments which are described in connection with the method. Especially preferable are, however, particles which are adapted for oral, parenteral, nasal or pulmonal administration of the active substance.

25 Furthermore, for the manufacture of pharmaceutical preparations for oral administration, the microspheres prepared by the method described may be formulated with an excipient (e.g. lactose, sucrose, starch etc.), a disintegrant (e.g. starch, calcium carbonate, etc.), a binder (e.g. starch, gum arabic, carboxymethylcellulose, 30 polyvinylpyrrolidone, etc.) and/or a lubricant (e.g. talc, magnesium stearate, polyethylene glycol etc.) and the resulting composition can be compression-molded in conventional manner. The particles can also be filled into gelatine capsules.

In the accompanying drawing figure the results of in vitro release tests are presented for particles obtained by the method of the present invention as well as particles obtained in line with the prior art.

5 The manufacture of said particles and the test method are described in Examples 1-5 and the results are presented as cumulative release in % versus time in days.

10 In this context it can also be added that the release profile can be controlled by factors well known to anyone skilled in the art, e.g. the composition of the polymer used for encapsulating the active material, the solubility of the material, addition of substances affecting the solubility of the active material and/or degradation of the polymer, the amount of active material 15 in the microparticles and the size of the microparticles.

EXAMPLE 1

20 The following procedure was used to encapsulate bovine serum albumin (BSA) in PLGA (poly(DL-lactide-co-glycolide)). First a polymer solution was prepared by dissolving 0.47 g of PLGA (RG504H, Boehringer Ingelheim) in 3 ml of ethyl acetate in a test tube. Then, 44 mg of BSA, (bovine serum albumin; Sigma A-0281) was dissolved in 300 μ l of 10 mM Na-phosphate buffer pH 6.4. The BSA 25 solution was added to the polymer solution and the BSA was homogenously dispersed in the polymer solution by vortex mixing (VF2, IKA-WERK) for one minute. The dispersion was placed in a 5 ml syringe with an 18 G needle.

30 A 500 ml beaker containing 300 ml of 40% (w/w) polyethylene glycol 20000 was fitted with a 4-bladed propeller stirrer. The BSA/polymer dispersion was transferred to the beaker by slowly injecting the BSA/polymer dispersion into the PEG solution. The stirrer speed was then reduced and the mixture was left standing overnight.

35 The stirrer speed was set at 8 again and then 400 ml of deionized water were added to reduce the viscosity in order to enable filtration. The suspension was then fil-

tered using a Millipore membrane filter, Type DV, pore size 0.65 μm , washed with water (3x 300 ml) and dried in vacuum overnight.

5 The resulting microparticles were spherical with a particle diameter of 10-50 μm and contained 6.3 % of BSA (w/w).

10 The resulting microparticles were then subjected to an in vitro release test in 30 mM sodium phosphate pH 7.4 at 37°C, with intermittent agitation. The studies were conducted by suspending 40 mg of microspheres in 1.5 ml of buffer. At specified time points, 1 ml of the buffer was withdrawn and replaced with fresh buffer. The results are shown in figure 1. Sustained release of BSA was achieved for 28 days as is shown in figure 1.

15

EXAMPLE 2

20 The same procedure was performed as in example 1 except that 2% (w/w) polyvinyl alcohol (PVA, mw=22000, Fluka) in water was used instead of the polyethylene glycol solution.

25 The resulting microspheres had a particle diameter of 1-2 mm and contained 7.0% of BSA. An in vitro release test was conducted as in example 1 and the results are shown in figure 1. Sustained release for about 2 days was achieved with this formulation. The large size would not have permitted injection using acceptable needles.

EXAMPLE 3

30 The same procedure was performed as in example 1 except that the an Ystral homogenizer was used instead of said stirrer when adding the BSA/polymer dispersion. After addition of the BSA/polymer dispersion the homogenizer was replaced by the 4-bladed propeller stirrer.

35 The resulting microspheres had a particle diameter of 1-5 μm and contained 5.5% of BSA. An in vitro release test was conducted as in example 1 and the results are shown in figure 1.

EXAMPLE 4

5 The same procedure was performed as in example 2 except that the an Ystral homogenizer was used instead of a stirrer when adding the BSA/polymer dispersion.

10 The resulting microspheres had a mean particle diameter of 10-40 μm and contained 5.8% of BSA. An in vitro release test was conducted as in example 1 and the results are shown in figure 1. Similar dissolution profiles 15 were obtained for the preparations in examples 3 and 4 even though the size of the particle in example 3 was much smaller.

EXAMPLE 5

15 The same procedure was performed as in example 1 except that an ultrasonic bath (Transsonic 470/H, Elma) was used after the vortex mixing in order to obtain a finer water-in-oil emulsion. The BSA/polymer dispersion was 20 sonicated for 1 minute.

20 The resulting microspheres had a mean particle diameter of 10-50 μm and contained 6.8% of BSA. An in vitro test was conducted as in example 1 and the results are shown in figure 1. Sustained release for 28 days was 25 achieved. This shows that a more efficient emulsification of the inner aqueous phase results in a lower rapid initial release (burst) during the first days.

EXAMPLE 6Preparation of BSA loaded microspheres

30 The following procedure was used to encapsulate Bovine Serum Albumin (BSA) in PLGA microspheres.

First a polymer solution was prepared by dissolving 0.126 g of polymer (Resomer 504H, Boehringer Ingelheim) with 0.734 of ethyl acetate in a test tube. Then 15 mg of 35 BSA (Sigma A-0281) were dissolved in 100 μl of 10 mM sodium phosphate pH 6.4.

The BSA solution was mixed with the polymer solution

by vortex mixing (VF2, IKA-WERK) for one minute. The solution was withdrawn into a 2 ml syringe with a 21G needle. A 200 ml beaker containing 50 ml of 40% (w/w) polyethylene glycol 20000 was fitted with a 4-bladed propeller stirrer. The BSA/polymer dispersion was slowly injected into the PEG solution during stirring at 240 rpm. The stirring speed was increased to 400 rpm for 10 seconds then the stirring speed was 60 rpm for one minute. The mixture was left standing unstirred for 4 hours.

10 200 ml of water were then added before filtration. The microsphere suspension was filtered using a Millipore membrane filter, Type DV, pore size 0.65 μm , washed with water and then freeze-dried overnight.

15 The resulting microparticles were spherical with a particle diameter of 10-50 μm and contained 9.7% of BSA (92% yield).

EXAMPLE 7

Preparation of Lactoglobulin loaded microspheres

20 The same procedure was performed as in example 6, except that 15 mg of Lactoglobulin (Sigma L-0130) in 100 μl 10 mM sodium phosphate pH 6.4 were used for encapsulation.

25 The resulting microparticles were spherical with a particle diameter of 10-100 μm and contained 9.9% of lactoglobulin (93% yield).

EXAMPLE 8

Preparation of Triptorelin loaded microspheres

30 The same procedure was performed as in example 6, except that 15 mg of Triptorelin pamoate (Bachem) were emulsified directly in the polymer solution by vortex mixing for one minute. The particle size of triptorelin particles was about 2-4 μm .

35 The resulting microparticles were spherical with a particle diameter of 20-100 μm and contained 6.3% of Triptorelin (59% yield).

EXAMPLE 9Preparation of Desmopressin loaded microspheres

5 The same procedure was performed as in example 6, except that 15 mg Desmopressin acetate in 100 μ l of 10 mM sodium phosphate pH 6.4 were used for encapsulation.

The resulting microparticles were spherical with a particle diameter of 10-50 μ m and contained 8.3% of Desmopressin (78% yield).

10

EXAMPLE 10Preparation of Insulin loaded microspheres

15 The same procedure was performed as in example 6, except that 15 mg Insulin (Sigma I-5500) were emulsified directly in the polymer solution by vortex mixing for one minute. The particle size of the insulin particles was about 5-10 μ m.

20 The resulting microparticles were spherical with a particle diameter of 10-50 μ m and contained 9.3% of Insulin (88% yield).

EXAMPLE 11Preparation of DNA loaded microspheres

25 The same procedure was performed as in example 6, except that 100 μ l of Herring Sperm DNA (Promega) (10 mg/ml) were used for encapsulation.

The resulting microparticles were spherical with a particle diameter of 10-50 μ m and contained 0.07% of DNA (10% yield).

30

EXAMPLE 12Preparation of Bovine Serum Albumin in 50% PEG 10k

35 The same procedure was performed as in example 6, except that 50% of PEG 10k was used as the external phase.

The resulting microparticles were spherical and contained 1.77% of BSA. This should be compared to 6.3% in

example 1.

EXAMPLE 13

Preparation of Bovine Serum Albumin in 30% PEG 35k

5 The same procedure was performed as in example 1 except that 30% of PEG 35k was used as the external phase. The resulting microparticles were spherical and contained 5.42% of BSA. This should be compared to a core load of 6.3% in example 1.

CLAIMS

1. A method of encapsulating an active substance in a biodegradable polymer, which comprises:
 - a) dissolving said biodegradable polymer in an organic solvent therefor;
 - b₁) dispersing said active substance in the organic solution obtained in step a), to provide a dispersion with the active substance as the inner phase thereof; or alternatively
 - 10 b₂) emulsifying said active substance, dissolved in water or other aqueous solvent therefor, in the organic solution obtained in step a), to provide an emulsion with the active substance as the inner aqueous phase thereof; and
 - 15 c) subjecting the dispersion obtained in step b₁), or alternatively the emulsion obtained in step b₂), to an encapsulation operation with an aqueous polyethylene glycol solution as a continuous phase, such that micro- or nanoparticles having the active substance encapsulated therein are obtained.
2. A method according to claim 1, wherein the microencapsulation operation in step c) is performed in the presence of an aqueous polyethylene glycol solution having a polyethylene glycol concentration within the range of 20-80% (w/w), preferably 20-60% (w/w), such as 30-55% (w/w) or 30-50% (w/w).
3. A method according to any one of claims 1 and 2, wherein the polyethylene glycol has a molecular weight of about 1000 to 40000 Da, preferably about 5000 to 35000 Da.
4. A method according to any one of claims 1, 2 and 3, wherein the encapsulation operation in step c) is performed by adding the dispersion obtained in step b₁), or alternatively the emulsion obtained in step b₂), to said aqueous polyethylene glycol solution while subjecting last-mentioned aqueous solution to a stirring and/or ho-

mogenization operation.

5. A method according to claim 4, wherein the stirring and/or homogenization operation is performed by a low intensity and/or low energy process, e.g. propeller 5 mixing or the use of motionless mixers.

6. A method according to any one of the preceding claims, wherein said encapsulation operation in step c) is performed in the absence of any surfactant.

7. A method according to any one of the preceding
10 claims, wherein said biodegradable polymer is insoluble,
or slightly soluble, in the aqueous polyethylene glycol
solution used in step c), preferably an aliphatic polyes-
ter.

8. A method according to any one of the preceding
15 claims, wherein said biodegradable polymer has a weight
average molecular weight in the range of about 2000 to
200 000, preferably about 2000 to 110 000.

9. A method according to any one of the preceding claims, wherein said biodegradable polymer is selected from homo or copolymers prepared from α -hydroxy acids, preferably lactic acid and glycolic acid, and/or cyclic dimers of α -hydroxy acids, preferably lactides and glycolides.

10. A method according to claim 9, wherein a copolymer of lactic acid/glycolic acid or a mixture of polylactic acid/polyglycolic acid is used as said biodegradable polymer, the weight ratio of (poly)lactic acid/(poly)glycolic acid being within the range of about 99/1 to 35/65, preferably 95/5 to 50/50.

30 11. A method according to any one of the preceding
claims, wherein said organic solvent used in step a) is
immiscible or essentially immiscible with said aqueous
polyethylene glycol solution used in step c), but
slightly or very slightly soluble therein, and capable of
35 dissolving said biodegradable polymer, and is preferably
selected from ethyl acetate, dichloromethane, methyl et-
hyl ketone and/or methyl isobutyl ketone.

12. A method according to any one of the preceding claims, wherein the active substance which is dispersed in step b₁) has a particle size within the range of about 0.5-20 μm , preferably 0.5-10 μm , more preferably 0.5-3
5 μm .

13. A method according to any one of the preceding claims, wherein said active substance is a biologically active substance, which is preferably selected from proteins, (poly)peptides, (poly)nucleotides, plasmides and
10 DNA.

14. A method according to claim 13, wherein said biologically active substance is selected from growth hormone, erythropoietin, interferon (α, β, γ -type), vaccine, epidermal growth hormone, Factor VIII, LHRH analogue, insulin, macrophage colony stimulating factor, granulocyte colony stimulating factor and interleukin.
15

15. A method according to any one of claims 1-12, wherein said active substance is a biologically active substance in the form of a non-protein drug selected from
20 the following groups:

anti-tumor agents, antibiotics, anti-inflammatory agents, antihistamines, sedatives, muscle relaxants, antiepileptic agents, antidepressants, antiallergic agents, bronchodilators, cardiotonics, antiarrhythmic agents, vasodilators, antidiabetic agents, anticoagulants, hemostatics,
25 narcotics, narcotic agents and steroids.

16. A method according to any one of claims 1-12, wherein said active substance is a non-biological substance, which is preferably selected from pesticide, fragrance, flavouring agent, catalyst and herbicide.
30

17. A method according to any one of the preceding claims, wherein the amount of said active substance is in the range of about 0.001% to 90%, preferably about 0.01% to 70%, more preferably about 0.1 to 45%, and most preferably about 0.1 to 40%, said percentage being by weight based on the weight of the final particles.
35

18. A method according to any one of the preceding

claims, wherein the particles obtained in step c) are separated from said continuous phase, preferably by centrifugation or filtration followed by rinsing with water or other aqueous medium, and dried or allowed to dry, for 5 instance in a vacuum, in the presence of a nitrogen gas flow, by lyophilisation or by air suspension drying.

19. A method according to any one of the preceding claims, wherein step c) is performed such that the particles obtained are microspheres or capsules or nanospheres or capsules.

20. A method according to claim 19, wherein said particles have a mean diameter in the range of 10-200 μm , preferably 20-100 μm .

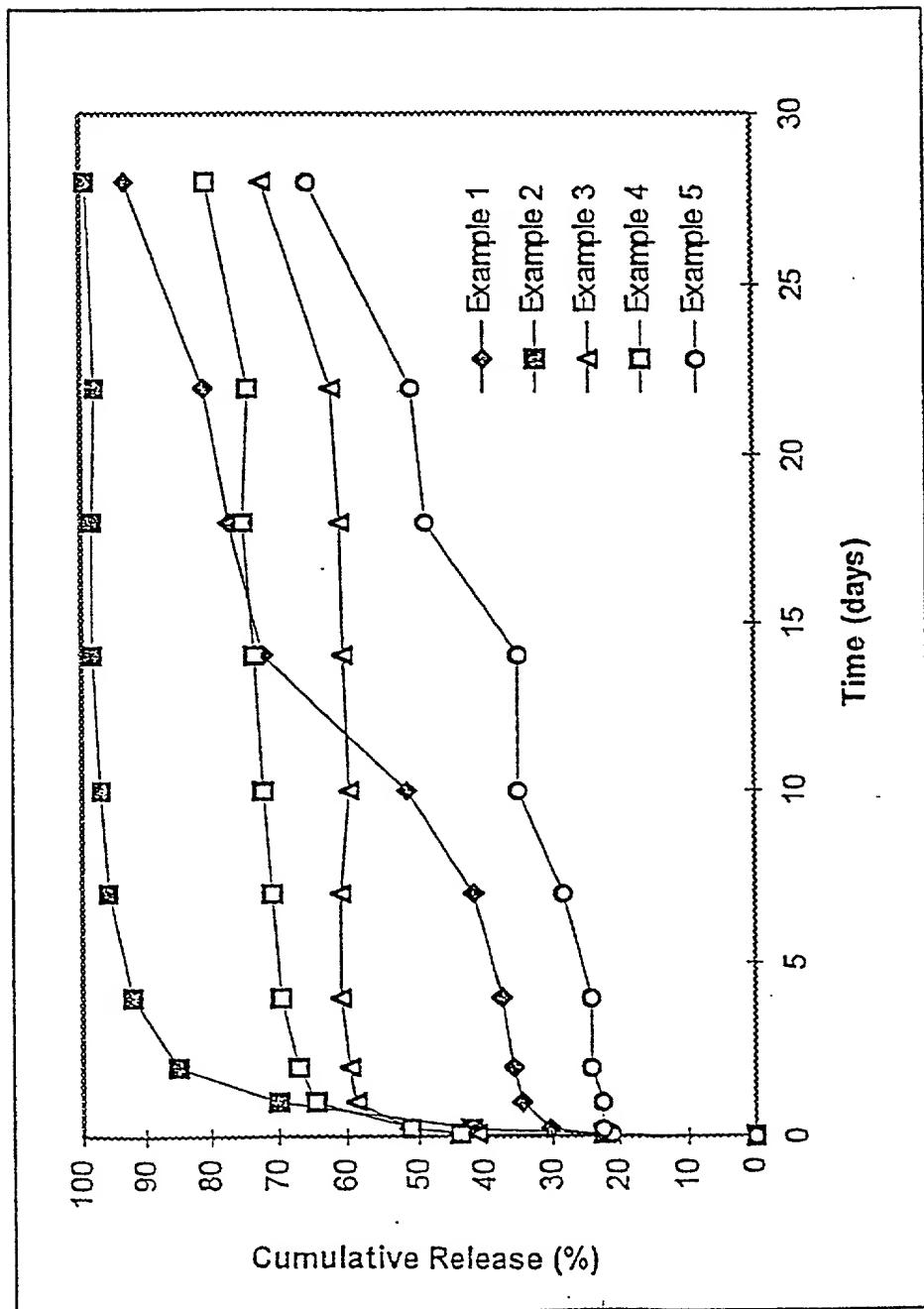
21. Sustained release micro or nanoparticles containing an active substance encapsulated in a biodegradable polymer, obtainable by a method according to any one of claims 1-20.

22. Particles according to claim 21, which are suitable for parenteral, nasal, pulmonal or oral administration of said active substance.

Abstract of the Disclosure

A novel method of encapsulating an active substance in a biodegradable polymer, which comprises: a) dissolving said biodegradable polymer in an organic solvent therefor; b₁) dispersing said active substance in the organic solution obtained in step a) to provide a dispersion with the active substance as the inner phase thereof; or alternatively b₂) emulsifying said active substance, dissolved in water or other aqueous solvent therefor, in the organic solution obtained in step a) to provide an emulsion with the active substance as the inner aqueous phase thereof; and c) subjecting the dispersion obtained in step b₁), or alternatively the emulsion obtained in step b₂), to an encapsulation operation with an aqueous polyethylene glycol solution as a continuous phase to provide micro- or nanoparticles having the active substance encapsulated therein. Sustained release particles obtainable thereby.

1/1

FIGURE

**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR UTILITY PATENT APPLICATION**

Attorney's Docket No.

003300-634

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (if only one name is listed below) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (if more than one name is listed below) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

ENCAPSULATION METHOD

the specification of which

(check one)

is attached hereto;

was filed on 24 September 1998 as

Application No. PCT/SE98/01717

and was amended on _____;
(if applicable)

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE;

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than twelve months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code Sec. 119 and/or Sec. 365 of any foreign application(s) for patent or inventor's certificate as indicated below and have also identified below any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application(s) on which priority is claimed:

COMBINED DECLARATION AND POWER OF ATTORNEY			Attorney's Docket No.
COUNTRY/INTERNATIONAL	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
Sweden	9703874-9	23-10-1997	YES ^X NO
			YES NO

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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